From pancreatic extracts to artificial pancreas: History, science and controversies about the discovery of the pancreatic antidiabetic hormone. I: The Pioneers

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Overview of main steps towards the discovery of the pancreatic antidiabetic hormone

Relevant scientific discoveries are usually based upon previous contributions of others. The careful review of original scientific papers and other documents regarding the chronological sequence of events leading to the isolation of the “antidiabetic hormone of the pancreas” allows concluding that the discovery was a multi-step process shared by many individuals and teams of investigators. Frederick G. Banting and his research director John J.R. Macleod were awarded the Nobel Prize for Physiology and Medicine in 1923.

Were these Canadian researchers the first ones leading to the discovery and preparation of the pancreatic antidiabetic hormone, known as insulin?

A critical review about the historiography of documented and successful initiatives to treat diabetes mellitus with pancreatic extracts is the main objective of series of articles to be published in “Avances en Diabetología”, the official journal of the Spanish Diabetes Society (SED). The present manuscript means the first one of them.

The Pioneers

In 1889, Oskar Minkowski and Joseph F. von Mering made a key observation, demonstrating that total pancreatectomy induced diabetes in the dog. In 1890, Minkowski already administered first dried fresh pancreas (called “pancreatine” by himself), and after observing its failure, he injected pancreatic extracts subcutane-
ously, also without effective response. Since then, many researchers decided to test the effects of pancreatic extracts in the treatment of animal and human diabetes. Their findings cannot be considered conclusive, due to incomplete developments and/or misinterpretations.

Most relevant pioneer works were carried out by Georg L. Zuelzer and Israel S. Kleiner. On June 21 and June 22, 1906, Zuelzer injected subcutaneously his pancreatic extract to a comatose adult diabetic subject who showed an impressive transient recovery. As there was no more extract, the patient went back into diabetic coma on June 30, and died 3 days later. He even registered a patent on acomatol, reporting beneficial effects on the excretion of sugar, and ketone bodies.1 In 1915, Kleiner and Meltzer demonstrated that in depancreatized dogs depicting marked hyperglycemia, the intravenous infusion of a pancreas emulsion brought the glucose content of the blood to normal levels.2 This relevant finding was confirmed by Kleiner by an extensive and comprehensive publication in 1919.3

**Pancreine**

Nicolas C. Paulescu, Professor of Physiology, University of Bucharest, published in a) the second volume of Traité de Physiologie Médicale (1920),4 in b) the July 23, 1921 issue of Compte Rendus de la Société de Biologie (Paris),5 and c) Archives Internationales de Physiologie (August, 1921),4 that he succeeded in obtaining an active extract from dog and beef pancreas, containing the antidiabetic hormone which he named pancreine. The injection of the extract into the external jugular vein was effective in lowering blood glucose in both pancreatectomized and normal dogs. The product also exhibited antiketogenic properties. Their biological actions could not be reproduced by intravenous injection of normal saline or by extracts from other organs. Oral or rectal routes were not effective. Paulesco even developed a partially purified extract for subcutaneous injection which he administered for the first time to a man on February 25, 1922, and to a few subjects afterwards. On April 10, 1922, the patent of pancreine was licensed by the Romanian Ministry of Industry.

**Iletin/Insulin**

Frederick G Banting and Charles H Best, from the Department of Physiology (Director, Professor JJR Macleod) first tried the duct ligation. After inducing atrophy of the exocrine pancreas, they obtained an extract which was able to reduce the hyperglycemia of pancreatectomized dogs. They also developed more active extracts from fetal and adult ox pancreas, without the need of duct ligation. The antidiabetic substance was named illetin first, and later renamed insulin, word that had been proposed for the first time by Jean B de Meyer in 1907. These results were presented by Banting and Best on November 14, 1921, before the Physiological Journal Club of the University of Toronto, and were first reported to the American Society of Physiology by Banting, Best and Macleod in December 1921. Finally, Banting and Best, published the leading article of their research in the Journal of Laboratory and Clinical Medicine (February 1922).7 The relevant results of this work were essentially the same than those previously reported by Zuelzer, Kleiner, and Paulescu.

**Purification of the pancreatic extract**

The collaboration of J B Collip allowed the purification of the extract. It was followed by wide use in the treatment of human diabetes. On January 23, 1922, the first successful administration of the new extract to a diabetic subject occurred. The first clinical trial was published in the Canadian Medical Association Journal on March 1922.8 On May 1922, the Board of Governors of the University of Toronto accepted the proposal to file the application for a Canadian patent in the names of Collip and Best for just the process of developing the insulin extract. On June 1922, the University of Toronto decided to file a request application in Best’s name on the antidiabetic product. In December 1922, the Collip/Best patent application was updated to include both the process and the product.

**Diabetes mellitus: a pancreatic disease**

The extraordinary influence of Claude Bernard as the creator of the scientific method in medicine made possible, in 1865, the development of the incipient Physiology and the birth of Endocrinology.9 Bernard elaborated the fundamental basis of glucose homeostasis, the role of the liver in glucose metabolism, as well as the intervention of the central nervous system in the induction of hyperglycaemia and glycosuria (diabetes as the consequence of the puncture of the floor of the fourth ventricle). Bernard also explored, although less successfully, the significance of the pancreas in diabetes. After observing that pancreatic atrophy, induced by ligation of pancreatic ducts, was not associated to diabetes in the experimental animal, Bernard abandoned the hypothesis of pancreatic diabetes10 (figure 2).

In his doctoral thesis of just 31 pages, dedicated to Rudolf Virchow, Paul...
Langerhans described “that in the pancreas, we have no direct transition of the secretory elements into the epithelia of the ducts”. The relevance of this observation was not taking in consideration until that Edouard Laguesse described the cell clusters as the islet of Langerhans, in 1983. In 1909, Jean de Meyer introduced the word “insulin” to describe the product of the islets of Langerhans.

In the year 1889, Oskar Minkowski and Joseph F. von Mering, when working at the Department of Medicine at the University of Strasbourg (directed by Bernard Naunyn) decided to remove the pancreas from a dog in order to know if the pancreatic enzymes were vital to the digestion of fat. Surprisingly, the animal developed polyuria and heavy glycosuria. This observation was confirmed in additional similar experiments demonstrating for the first time that the pancreatectomised dog develops diabetes mellitus; i.e. the absence of pancreas causes diabetes. The vision of these events and statements of Professor B. Naunyn (Strasbourg) about main protagonists were subjects of a particular article by the Nobel Prize Bernardo Alberto Houssay. “Undoubtedly, the discovery of pancreatic diabetes was due to Minkowski’s determination and technical dexterity; it was he who removed the pancreas from a dog and found sugar in its urine... He was also fair to von Mering and associated him in the publication of the results as was his due... The true fact is that von Mering did not discover pancreatic diabetes nor did he do research in this field after his first publication with Minkowski” (B. Houssay). Among several others, Emmanuel Hédon, working in Montpellier (France), reproduced diabetes in the pancreatectomy model, carried out in two steps, preserving the procesus uncinatus in the first one, which prevented the development of diabetes until the final exeresis of this remnant in the second step.

Before the discovery of Minkowski and von Mering, Etienne Lancereaux had introduced in 1877 the concept of pancreatic diabetes after describing two young severe diabetic subjects who died and showed advanced atrophy of the pancreas. As referred by C. Ionescu-Tirgoviste “Speculations as to the role of the pancreas in diabetes between 1850 and 1877, are numerous. However, the evidence was not enough to convince all since in the vast majority of diabetic patients the pancreas was of normal size and appearance at autopsy”. For researchers like Eduard Pflüger, the hyperglycemia could be the consequence of injury to the celiac plexus during the operation, opposing the thesis that the pancreas is the site of diabetes. In favour of the pancreatic theory is the critical confirmation that ligature of the pancreatic excretory ducts is followed by atrophy of the acinar tissue but not of diabetes, suggesting that the pancreas must contain an antidiabetic substrate. Minkowski implanted pancreas subcutaneously, and Hédon undertook successful transplants, allowing the conclusion that the pancreas secreted an internal substance, essential for the maintenance of normoglycemia. In 1906, Wilhelm Heiberg developed in Copenhagen a method for counting the islets of Langerhans and found that the count was consistently low in diabetic subjects.

The next question was how the pancreas regulated carbohydrate metabolism. Minkowski was the first researcher in trying, unsuccessfully, to restore the antidiabetic function of the pancreas by administering pancreatic extracts orally and parenterally. If the pancreatic extract reduced glycosuria might content the internal secretion of the pancreas. Until this
Treatement of diabetes with pancreatic extracts (1890-1919)

Minkowski made glycosuria to disappear in depancreatized dogs by subcutaneous implants of pancreatic tissue. Early experiments with pancreatic extracts tended towards negative results with some exceptions. In a group of cases, the extracts were totally ineffective. In many occasions, they induced harmful effects. In other situations, transient beneficial effects on glycosuria were overcome by toxic effects. And finally, encouraging results in some cases were not reproduced by other authors.19

In 1892, Capparelli used an extract obtained by trituration of fresh pancreas in saline solution, and injected it into the abdominal cavity of a pancreaticomized dog. A substantial reduction of glycosuria was observed. For some authors, the correct interpretation of the results could be explained by just the partial extirpation of the pancreas.20 Eugène Glay, first in 1892, and afterwards in 1902, was able to induce experimental diabetes by ligation of the pancreatic venous system. Glay developed a successful pancreatic extract with positive results in his model of pancreatic diabetes. Curiously enough, he sent the results of his experiments to the Society of Biology in Paris, with the request of waiting to 1922 to unveil the content of the sealed package keeping the report of his unpublished results.

John Rennie and Thomas Fraser

J. Rennie and T. Fraser, from Aberdeen Royal Infirmary, investigated the effects of the islets of Langerhans of Lophius piscatorius and other teleost. These particular fishes like catfish, tuna, flounder, cod, monkfish, herring, salmon, etc, show the islets to be independent of proper pancreas, anatomically distinct from the acinar tissue. Even a particular islet, the so called “principal islet” is of relative large size, facilitating convenient supplies.

Between 1902 and 1904, Rennie and Fraser administered fish islet extracts to diabetic subjects. Islets were macerated in a mortar and digested for some time at 40°C, acidified (weak acetic acid), and filtered. Patients were on ordinary diet, except starch, and took the islets three times daily between meals. Urine sugar was estimated (Pavy/yeast method). The two Scots researchers from Aberdeen reported 5 cases. The first patient was male, 18 years old, almost blind. He received an average daily dose of 0.6 g of islets. Urine sugar did not show much fluctuation. Patient was treated from November 1902. After March 23, 1903, supply of islets failed and he died on April 3. The second case was female, 45 years old; she received a daily dose, about 1.5 g, with negative results. The third case was male, 44 y old. He received also 1.5 g of islets preparation, orally, at different intervals. Later on, the extract was given hypodermically, without success. The patient died on diabetic coma. Case 4 was male, 50 years old. He received boiled extract, although only small quantities were available; left the hospital and declined to remain longer under treatment. The last case was a 59 year old woman, almost asymptomatic but depicting heavy glycosuria. Disappearance of urine sugar was observed after a few days of active treatment. Nevertheless, the interpretation given was that this effect was probably the consequence of the strict diet administered at the same time.21

Georg Ludwig Zuelzer

Zuelzer initiated his studies on pancreatic extract with the main assumption of an antagonism between the function of the adrenals and the pancreas, after the description of Blum in 1901 of adrenalin-diabetes. In his laboratory, he used rabbits, observing that the injection of at least 1 ml of adrenalin regularly induced hyperglycaemia and glycosuria. Then, he demonstrated a preventive effect of glycosuria in rabbits injected with a preparation of adrenal glands, by the administration of an alcoholic pancreatic extract. He measured the potency of the pancreatic extracts by the amount of extract needed to neutralize the hyperglycaemia secondary to the administration of one unit of adrenalin. Zuelzer also investigated the reduction of urine glucose excretion in depancreatized dogs after the ligature of the adrenal venous effluent.22 In other multiple experiments, Zuelzer observed decreased glucose excretion of depancreatized dogs, and also in diabetic subjects, after the administration of pancreatic extracts, obtained by various methods. When he interrupted the administration of the extract, the urine glucose excretion returned to previously elevated levels.

On June 17, 1906, Zuelzer administered, for the first time, a subcutaneous injection of 8 cc of a solution containing 3 g. of pancreatic extract to a 50 year old diabetic subject, known suffering the disease from at least 3 years. Zuelzer worked with calves’ pancreas, removed at the height of the digestion, after ligature of the veins for one hour with the in-
tention to achieve maximally optimal accumulation of the active substance. The patient was presented to the Clinic with a picture of diabetic gangrene and major amputation below the left knee, which was performed on June 10. A second injection of the pancreatic extract was given subcutaneously on June 18, 1905. It was a dose of 5 g dissolved in 10 cc of water. In spite of an apparent clinical improvement, the patient died on July 2, after entering in deep coma on June 30. Additional doses of pancreatic extracts were not available.

The effects of the pancreatic extracts on a second diabetic patient were as it follows. He was a 27 year old subject with diabetes diagnosed in February, 1905. He suffered also pulmonary tuberculosis. He was admitted at the hospital on May 27, 1907, with polyuria, urine volume above 4 l/24 hours, and glycosuria of 6%. Urinary determinations of acetone and acetoacetic acid were positive. On June 26, the first experimental dose (1 ml) of the pancreatic extract was given intravenously. A second injection of 9 ml containing 2 g of pancreas extract was administered on July 1, 1908. A marked reduction of urine glucose excretion was recorded on July 2 and on July 3, in which only traces were depicted. The disappearance of ketone bodies in the urine was also evident. On July 4, glycosuria increased and ketonuria was restarted. On July 6, the chemistry determinations returned to the previous status. Additional doses of different pancreatic extracts were given afterwards without success. The author argued about the ineffective action of these extracts.

A peculiar case was a 6 year old child, seriously ill, admitted with a picture of malnutrition, glycosuria and ketosis. On July 14, 1907, 5 ml of the emulsion containing 1 g of pancreatic extract were injected in an antecubital vein. Body temperature raised immediately after the injection to 38.4 °C, followed by vomits. On July 16, the general clinical situation improved. Ketonuria disappeared for 48 hours, remaining urine glucose excretion without much change. A weight increase of 13.4 kg was recorded. On August 1, 1907, an injection of 1 g of the dry extract in 3.5 ml was given again. Temperature raised to 39.2 °C. Urine glucose excretion was reduced somehow and ketone bodies almost disappeared. After 48-72 hours, the patient parameters returned to the previous levels. Unfortunately, the male child was discharged soon after and died. The clinical impression indicated a favorable, although temporary, effect of the pancreas extract on the patient’s condition.

Another case was a 65 y old patient with diabetes since the age of 39. On July 15, 1907, 10 ml of the emulsion containing 2 g of the dry pancreatic extract were intravenously administered on July 1, 1908. A marked reduction of urine glucose excretion was recorded on July 2 and on July 3, in which only traces were depicted. The disappearance of ketone bodies in the urine was also evident. On July 4, glycosuria increased and ketonuria was restarted. On July 6, the chemistry determinations returned to the previous status. Additional doses of different pancreatic extracts were given afterwards without success. The author argued about the ineffective action of these extracts.

In 1908, Zuelzer gave the name of ACOMATOL to the pancreatic extract. In 1909, following Minkowski advice, J. Forschbach repeated Zuelzer’s clinical experiments in the Minkowski Clinic, in Breslau. He noticed that glycosuria decreased. Forschbach decided to stop human treatment, after observing side effects in two patients, especially severe fever, attacks of shivering, racing pulse, vomiting and sweating. Nevertheless, Forschbach confirmed the antidiabetic action of the pancreatic extract itself: “First (GL Zuelzer) to produce, successfully, from the pancreas a preparation that eliminates sugar excretion in a shorter or longer period by iv administration”.

Because of the discouraging results of Forschbach, the Schering Co. withdrew the financial support provided to Zuelzer. Also, the University of Berlin rejected to provide Zuelzer a grant to spend 6 weeks at a zoological station to intend making an extract from teleost fish pancreas. Nevertheless, in 1911 Hoffman – La Roche funded him a small laboratory and helped to obtain an USA patent on Zuelzer’s pancreas preparation, suitable for the treatment of diabetes mellitus (Licensed USA patent...
The Zuelzer’s method of pancreatic extract using alcohol extraction and distilled protein precipitation in a low temperature vacuum seems to have been a very effective preparation. The laboratory of the firm Hoffman La Roche, directed by Camille Reuter, was able to process up to 114 kg of pancreas. The animals tested, mainly dogs, developed severe convulsions, never seen before by Zuelzer, who postulated that a foreign substance had gained access to the preparation. Reuter attributed it to the copper vessels used in the procedure. Actually, the animals had received an excessive amount of what we call now insulin, responsible for the induction of hypoglycemic shock. Unfortunately, Zuelzer did not realize the fact that convulsions were due to hypoglycaemia. He did not estimate blood sugar content in those experiments.

On August 1914, the experiments succeeded to the extent that an extract could be prepared, which after iv injection reduced the blood sugar to 17 mg/dL. The disadvantage was that the effect lasted only a few hours, and injections every 3 hours were needed to maintain the action. Thus, Zuelzer was the first to use an effective pancreatic extract in both animal experimentation and treatment of human diabetes. Quite soon after the Nobel Prize award to FC Banting and JJR Macleod, Zuelzer declared in Medizinischen Klinik (1923;47:15551-1552): “I am now entitled to state my claim to priority in this discovery…because in the German literature, partially from ignorance, the role that fell to me in the discovery was not always perceived quite correctly”. Zuelzer, a Jewish refugee from Nazism, emigrated to USA in 1934, and spent the last years of his life practicing medicine. He died in a nursing home in New York.

Frederick Madison Allen

FM Allen introduced the starvation diet for diabetic patients. Patients were starved until disappearance of glycosuria and then put on a hypocaloric diet, with a very poor carbohydrate intake. He also performed experimental research, removing about 90% of the pancreas, generating a mild diabetes. He tried, unsuccessfully, the administration of pancreatic extracts. In 1913 declared that all pancreatic preparations had been useless and harmful. Later on, he established the Physiatric Institute in Morristown, New Jersey. The dietary treatment was the best option for patients at those years.

Ernest Lyman Scott

E.L. Scott from the Department of Physiology at the Columbia University was influenced by previous reports of Leschke (1910) and Hédon (1911). Those experiences have suggested that the external digestive enzymes of the pancreas have a deleterious effect upon the experimental animal. Attempts to destroy these enzymes by heat destroyed the internal secretion as well. It was hoped that the presence of digestive enzymes could be eliminated by the atrophy of the gland which follows complete ligation of the ducts. Nevertheless, after various attempts in the dog, the method was abandoned as impractical. Therefore, in subsequent work these enzymes were intended to render inactive by a high percentage of alcohol. The glands were extracted for three twenty-four hour periods at 35-40 degrees Celsius. Then, filtered and evaporated to dryness, and the residue extracted with ether at room temperature. After discarding the ether extract, the residue was dissolved in 95% alcohol. After new evaporation, the extract was dissolved in saline solution and ready for injection.

Scott also prepared watery extracts. After handling the alcoholic extracts as above, the material was evaporated to dryness under a vacuum and extracted in absolute alcohol at 38 degrees. After pouring off, the resi-
due was extracted with water rendered acid by acetic acid. Then, the preparation was filtered and evaporated. Before injecting, the ethanol was eliminated and salt solution added. In general, the intravenous injection of the pancreas extract into pancreatectomized dogs diminished temporarily the excretion of sugar. The interpretation of Scott was that the pancreas extract could reduce the output of sugar by a toxic action, rather than therapeutic.24

Scott performed also additional experiments with cats. The pancreatic extracts were prepared as before, except that the temperature was at all times kept below 50ºC instead of 65ºC. Five cats were injected with the extract, beneath the skin of the back. The administration of the pancreatic extract was followed by a surprising increase over 21% in blood glucose levels, above the normal cats. A logical explanation for this result could not be provided at that time.25

John Raymond Murlin

John R Murlin and Benjamin Kramer from the Laboratory of Physiology at the Cornell University of New York treated completely depancreatized dogs, following the Hédon’s method (1911), with intravenous injection of a pancreatic extract prepared as by Knowlton and Starling (1912). Urine was collected at short intervals after injection. A marked fall in sugar output was witnessed, lasting 4-10 hours. This was followed by a compensatory rise. With a double extract of dog’s pancreas and duodenal mucosa, much greater effects were observed. Nevertheless, similar results were observed using the same Ringer’s solution in which the tissues were extracted when made alkaline. The original interpretation given by the researchers was that the reduction in glucose excretion was due to a change in the permeability of the kidney, and not a hormonal effect.26 Years later, the authors reproduced the experiment with success, observing disappearance of glycosuria in conjunction with decline in blood glucose. Then, it was a change in the interpretation, suggesting that the active principle was present in the pancreatic extract.27

Israel S Kleiner

IS Kleiner and SJ Meltzer from the Department of Physiology and Pharmacology at the Rockefeller Institute for Medical Research investigated the effects of an intravenous injection of an emulsion of pancreas in normal and depancreatized animals exposed to large quantities of intravenous dextrose. Their findings were published in 1915. They observed in normal animals that ninety minutes after the infusion the dextrose content of the blood reached nearly the same level which it had before the injection. On the contrary, in non-treated depancreatized animals, the dextrose content of the sample blood taken ninety minutes after the end of the dextrose injection was at least twice as high as that of the sample taken before the injection. In a third group of experiments, a strained pancreatic emulsion was added to dextrose and intravenously administered. In such experiments, ninety minutes after the end of the infusion, the dextrose content of the blood was at the same level as it was before the infusion of dextrose, suggesting that the internal secretion of the pancreas contributed to the rapid disappearance of dextrose from the circulation.28

Kleiner reported in 1919 a group of experiments, carried out between 1915 and 1919, supporting the evi-
dence for the internal secretion of an antidiabetic pancreatic substance. He administered intravenous injections of unfiltered water extracts of fresh pancreas, diluted with 0.9 per cent NaCl solution, to normal, non-treat-
ed, and treated depancreatized dogs. Blood was drawn from an external yugular vein. Glucose concentration was measured by the Myers-Bailey procedure (1916), a modification of the Lewis-Benedict method requiring much smaller samples. Urine samples were obtained by catheter, and excreted glucose was estimated by either, a modification of the Pavy method or by the Benedict’s method (1911). The pancreatic emulsion granted an important reduction of blood glucose levels in ten out of sixteen experiments. The decrease in blood sugar was not accompanied by any reduction of haemoglobin levels, indicating that the effect was not due to dilution. Also, the kidney excretion of sugar experienced a great re-
duction, with the exception of one experiment. It is important to empha-
size that no alkali was used in preparing the pancreas emulsions. Ex-
tractions were made with water and the dilutions of the extracts with 0.9% NaCl. In control experiments, the animals received intravenous emulsions of submaxillary glands. The slight decrease in blood sugar observed was in mark contrast with the results achieved when pancreas emulsion was used, and evolved parallel with haemoglobin (dilution by intravenous injection of over 100 ml of fluid). Kleiner did not observe much toxicity of the pancreatic ex-
tracts, except occasional changes in body temperature. The explanation seems to be that the extract was highly diluted and very slowly injected. At the end of the general discussion of the manuscript, Kleiner said :”The fact that these pancreas emulsions lower blood sugar in experimental diabetes without causing marked tox-
ic effects indicates a possible thera-
peutic applications in human be-
ings...Finally, the search for the effective agent or agents, their purifi-
cation, concentration, and identification are suggested as promising fields for further work.” Unfortunately, Kleiner did not continue this area of research after leaving the Rockefeller Institute in 1919.

References